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Differential Regulation of PAI-1 in Hantavirus Cardiopulmonary Syndrome and Hemorrhagic Fever With Renal Syndrome

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We analyzed the levels of circulating tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)-1 in acute hantavirus cardiopulmonary syndrome (HCPS) and hemorrhagic fever with renal syndrome (HFRS). The levels of tPA commonly increased in both diseases, whereas PAI-1 correlated with disease severity in HCPS but not in HFRS.

Keywords. hantavirus; HCPS; HFRS; PAI-1; tPA.

Hantaviruses cause two diseases: hantavirus cardiopulmonary syndrome (HCPS) and hemorrhagic fever with renal syndrome (HFRS). The hallmark of both diseases is increased vascular permeability [1, 2]; however, the most affected organ generally varies from the lungs in HCPS to the kidneys in HFRS. The type of hantavirus disease is determined by the causative species. Andes (ANDV) and Sin Nombre (SNV) hantaviruses cause HCPS with mortality that can reach 40% in South and North America, respectively. Puumala hantavirus (PUUV) causes a mild HFRS, also called nephropathia epidemica (NE; mortality ~0.1%), mainly in Northern Europe and Russia, whereas Dobrava (DOBV) and Hantaan (HTNV) viruses cause severe HFRS (mortality 1%–5%) in the Balkans and East Asia, respectively [3].

Hantavirus infection activates intravascular coagulation pathways in the acute stage of HCPS and HFRS [4–6]. At the same time, however, PUUV has been suggested to cause enhanced fibrinolysis [5], that is, excessive solubilization of blood clots, which we recently showed to be characterized by elevated activity of circulating tissue plasminogen activator (tPA), whereas its main physiological inhibitor, plasminogen activator inhibitor-1 (PAI-1), remains unaltered [7]. In this study, we assessed the levels of tPA and PAI-1 in HCPS and HFRS caused by ANDV and DOBV, respectively, to better understand the role hemostatic regulation in hantavirus pathogenesis in general.

METHODS

The study protocol was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki and was approved by the Ethical Committee in Research of ANLIS “Dr C. Malbrán,” Slovenian National Medical Ethics Committee, and the Ethics Committee of the Tampere University Hospital for serologically confirmed ANDV, DOBV, and PUUV cases, respectively. The study cohort consisted of patient samples that were collected ≤10 days after the beginning of the disease, that is, after onset of fever. Severe HCPS was classified, for example, due to pulmonary failure and need of mechanical ventilation, whereas signs of lower respiratory compromise were considered mild HCPS. Non-HCPS cases with pulmonary complications were included as reference. The DOBV patients were classified as severe or mild, for example, based on the need for dialysis. Additional information on patient demographics and disease classification criteria can be found in [Supplementary Table 1](#) and the Supplementary Methods, respectively.

Protein levels of tPA and PAI-1 were quantitated from patient plasma or serum by enzyme-linked immunosorbent assays, similar to methods described previously [7].

RESULTS

The levels of circulating tPA were significantly elevated above control in both severe and mild acute HCPS but not in symptomatic non-HCPS cases ([Figure 1A](#)). Furthermore, tPA was significantly increased in severe as compared with mild HCPS. Its main physiological inhibitor, PAI-1, was also found to be increased above control in severe HCPS. However, in contrast to tPA, PAI-1 was not elevated in the mild HCPS or non-HCPS groups ([Figure 1B](#)). Thus, simultaneously elevated circulating levels of tPA and PAI-1 distinguish severe HCPS from mild HCPS and symptomatic non-HCPS cases. Strikingly, when taking into account all HCPS cases in our cohort, a robust correlation between fatality and PAI-1 ($r = .514$; $P = .001$), but not tPA ($r = .262$; $P = .1$), was observed.

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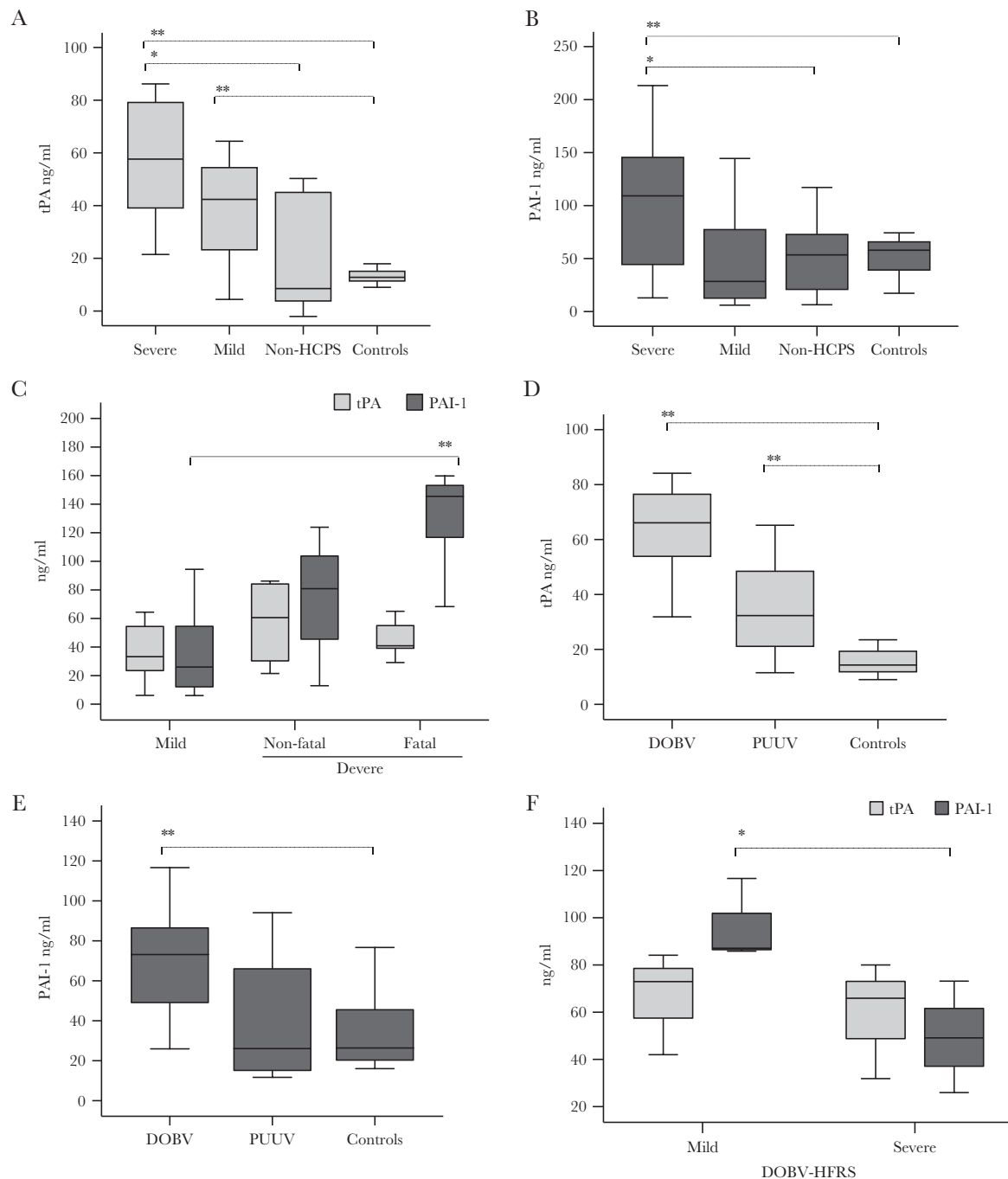


Figure 1. Levels of tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)-1 in acute hantavirus cardiopulmonary syndrome (HCPS) and hemorrhagic fever with renal syndrome (HFRS). Box plots of the levels of tPA (A) and PAI-1 (B) in acute (≤10 days after onset of disease) serum samples of severe (n = 18) and mild (n = 22) Andes (ANDV)-caused HCPS, non-HCPS cases (n = 5 for tPA and n = 12 for PAI-1) with similar symptoms to HCPS, and healthy volunteers (n = 10) as controls. (C) The levels of tPA and PAI-1 in early acute (≤5 days after onset of disease) serum samples of fatal (n = 7), severe but nonfatal (n = 4), and mild (n = 15) ANDV-caused HCPS. The levels of tPA (D) and PAI-1 (E) in acute (≤10 days after onset of disease) plasma samples of Dobrava (DOBV)- (n = 7) and Puumala hantavirus (PUUV)-caused (n = 16) HFRS in addition to healthy volunteers (n = 15) as controls. (F) The levels of tPA and PAI-1 in acute plasma samples of mild (n = 4) and severe (n = 3) DOBV-caused HFRS. Box plots illustrate the median value (center horizontal line), interquartile range (the lower and upper quartiles), and the highest and lowest values (whiskers) that are not outliers. Distributions across groups were compared by the Mann-Whitney *U* test. **Statistically highly significant difference at *P* < .01. *Statistically significant difference at *P* < .05.

To get further insight into the upregulation kinetics and the role of tPA and PAI-1 in HCPS disease severity, we further classified severe HCPS to fatal and nonfatal cases at early acute stage (≤5 days after onset of fever) (Figure 1C)

and found either statistically significantly or close to significantly elevated levels of PAI-1 in fatal HCPS as compared with mild (*P* = .001) or severe but nonfatal (*P* = .073) cases, respectively. tPA levels did not differ between groups. These

findings suggest a direct role for PAI-1, but not tPA, in the fatality of HCPS.

The levels of tPA and PAI-1 were also compared in the plasma of acute DOBV- and PUUV-caused HFRS patients. As seen in Figure 1D, the median levels of tPA were increased significantly above control in HFRS irrespective of the causative virus, but were further elevated by DOBV as compared with PUUV. While PAI-1 was not elevated in PUUV-HFRS (Figure 1E), it was significantly increased above control in DOBV-HFRS. This finding suggests a marked difference in regulation of PAI-1 based on the etiology and/or severity of HFRS. Interestingly, however, when categorizing DOBV-HFRS to mild vs severe forms of the disease based mainly on the extent of kidney failure and need for hemodialysis, PAI-1 was significantly lower in severe DOBV-HFRS, whereas no change between groups was observed for tPA.

These results indicate marked differences between the severe forms of HCPS and HFRS in the regulation of PAI-1. Further corroborating this difference, tPA was found to strongly correlate with PAI-1 in HCPS ($r = .471$; $P = .002$) but not in DOBV-HFRS ($r = .402$; $P = .371$). No correlation was observed between tPA or PAI-1 with platelet counts in HCPS or patient age in HCPS or HFRS (data not shown).

DISCUSSION

The results of this study show for the first time that circulating levels of tPA are increased in acute HCPS and HFRS caused by ANDV and DOBV, respectively. In addition, higher tPA levels were found in DOBV- vs PUUV-caused HFRS and in severe vs mild HCPS, indicating a possible role for tPA in hantavirus pathogenesis. This is in accordance with our previous study, where tPA was found to positively correlate with hemorrhages in PUUV-HFRS [7]. However, no significant differences in tPA levels were observed between severe vs mild DOBV-HFRS or fatal vs nonfatal severe HCPS, indicating that additional factors are necessary to fully explain virulence in both diseases.

In contrast to tPA, its main physiological inhibitor, PAI-1, was only increased in severe HCPS and generally in DOBV-HFRS. Furthermore, PAI-1 levels were nearly statistically significantly elevated in fatal vs nonfatal severe HCPS, indicating a possible role for this protein in the mortality of HCPS. This latter finding is in accordance with a previous report indicating higher plasma PAI-1 levels in fatal SNV-caused HCPS as compared with nonfatal cases [4]. However, seemingly contradicting the possible role of PAI-1 in the pathogenesis of HFRS, it was reduced in severe vs mild DOBV-HFRS (Figure 1F) and not elevated at all in PUUV-HFRS (Figure 1E) [7].

Due to differences in blood sampling (serum vs plasma), we were not capable of reliably comparing the quantity of PAI-1 between HCPS and HFRS cases. Platelets, which are activated during blood clotting in the preparation of serum samples,

are one of the major physiological sources of PAI-1 [8]. This affects baseline PAI-1 levels, as indicated by the increased concentration of PAI-1 in the serum of healthy volunteers (compare Figure 1B and E). On the other hand, this effect is probably negligible in HCPS patient serum due to the often severe thrombocytopenia associated with the disease [9], and thus the difference between PAI-1 levels in HCPS patients and controls in circulation is most likely higher than estimated in this study. In fact, thrombocytopenia might explain the slightly lower level of PAI-1 in mild HCPS vs healthy controls (Figure 1B). Another potential caveat in our study is the fact that all severe HCPS cases were, by definition, subjected to mechanical ventilation, which has the potential to increase the circulating levels of PAI-1 [10]. However, while we are not aware whether the analyzed samples were acquired before or after mechanical ventilation, we did not observe any changes in tPA or PAI-1 levels in non-HCPS cases receiving similar treatment as compared with HCPS cases (Figure 1A and B). Furthermore, by comparing fatal with nonfatal mechanically ventilated HCPS cases (Figure 1C), we observed a difference in PAI-1 levels, suggesting that ventilation per se is not a significant factor in the regulation of PAI-1 in HCPS. This is also in concordance with another study indicating that PAI-1 levels were increased in fatal vs nonfatal SNV-HCPS cases receiving mechanical ventilation and/or extracorporeal membrane oxygenation [4]. All severe DOBV-HFRS cases received hemodialysis, which also potentially affects hemostasis [11]. However, all DOBV samples were acquired before hemodialysis, eliminating the influence of this factor in the observed results.

Taken together, it is possible that the observed increase in tPA/PAI-1 ratio accounts for elevated hemorrhagic complications and kidney failure in severe as compared with mild DOBV-HFRS. This is in line with our previous findings, where tPA, in the concurrent absence of neutralizing PAI-1, correlated with hemorrhages in PUUV-HFRS [7]. Analogously, the highly elevated levels of PAI-1 could also explain the absence of hemorrhages in severe HCPS [12], which is in striking contrast to severe HFRS cases [9]. Minor hemorrhages were reported in 20% of our HCPS patients, but no correlations between bleeding and disease severity, tPA, or PAI-1 were found (data not shown). Further studies on the role of tPA and PAI-1 in hantavirus diseases are certainly warranted.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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